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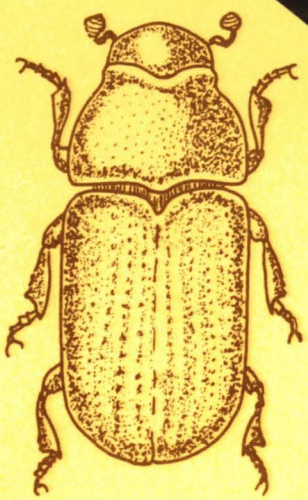
BIOLOGICAL EVALUATION
Mold Affecting Laboratory Germination of Range Shrub Seeds

Region 3

February 1980



Southwestern Region

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INTRODUCTION

Personnel from the Rocky Mountain Forest and Range Experiment Station's Mine Spoil Reclamation unit are testing the effects of temperature and moisture on germination of range shrub seeds as part of the Surface Environment and Mining (SEAM) program. The procedure for germinating seeds is as follows: vermiculite is placed in a plastic dish and covered with blotter paper; seeds are placed on the blotter paper, and 25 to 40 ml of tap water are poured over the seeds; dishes are closed with a tight fitting lid and subjected to various treatments. Molds are interfering with the germination experiments by invading dishes and killing the seeds. We determined the major sources of contamination, identified the causal organisms, and tested various seed coat treatments. This biological evaluation suggests alternatives which will provide adequate protection from mold without affecting other experimental conditions.

MATERIALS AND METHODS

A. Sources of contamination were identified by: (1) exposing potato dextrose and malt agar plates to the air while 50 seeds were being counted and placed in dishes with vermiculite and blotter paper; (2) placing vermiculite, untreated blotter paper, Thiram and Zineb-treated blotter paper, and unsterile seeds on agar plates; and (3) surface-sterilizing seeds by immersing them in 1 percent sodium hypochlorite, rinsing them in sterile distilled water, and plating them. Cultures were incubated at room temperature for up to 3 weeks. The cultures were examined regularly and all molds were identified.

B. In order to test the various methods of surface-sterilizing, the seeds and other materials, including blotter paper, vermiculite, dishes, and water, were sterilized and stored under a hood. Fifty seeds were counted in the open air, and then treated by: (1) rinsing under tap water for 1 hour; (2) surface-sterilizing by immersing seeds in 1 percent sodium hypochlorite for 30 seconds and rinsing in sterile distilled water; (3) pouring 25 to 40 ml of 0.1 percent Nystatin in water over seeds and into dishes; and (4) immersing seeds for 30 seconds in 25 to 40 ml of 0.1 percent Nystatin before pouring seeds and solution into dishes. The germination test procedures normally used were followed, and each treatment was replicated three times. Twenty-five to 40 ml of sterile distilled water were added to dishes with seeds subjected to treatments 1 and 2. Dishes were prepared under a simple hood.

RESULTS AND DISCUSSION

A. Table 1 shows the organisms isolated and their sources. The lab environment is the most common source of contamination of the seeds in the germination dishes, and the probability of contamination increases with time of exposure. The air coming from a ceiling vent contained molds. Normally a fan is placed in an open door while seeds are being counted in order to reduce the temperature of the lab. Higher levels of contamination would probably have resulted if this were done during our tests.

Sage seeds (Artemisia tridentata) appear to be free of surface contamination, but all the agar plates exposed while the sage seeds were counted became contaminated because the seeds are small and take a long time to count, exposing them for a longer time to the contaminated laboratory air. Mormon tea has large seeds and a shorter counting time, and therefore are exposed to the air for a shorter period. One agar plate out of five exposed during the time Mormon tea was being counted became contaminated.

Vermiculite and blotter paper are not sources of contamination, but harbor molds because they are exposed to lab air. Thiram and Zineb are effective against contamination from air, but mold is found after seeds and water are added, probably because of fungi on the seed.

B. Species of mold varied with the species of seed. No mold was found on sage seed. Shadscale (Atriplex confertifolia) had low levels of surface contamination which were eliminated by sodium hypochlorite. Two cultures of Rhizopus arrhizus [1/] were isolated from untreated winterfat (Certoides lanata). No R. arrhizus was isolated from the air and only one culture of R. arrhizus was isolated from both untreated vermiculite and blotter paper. All untreated Mormon tea seeds contained mold, mostly R. arrhizus. Mormon tea seeds appear to be the major source of R. arrhizus contamination in the lab, with the fungus originating from within the seed, as well as from the surface. Winterfat's pubescent seed coat renders surface sterilization ineffective; as a result, a wide variety of fungi were found on this species.

Table 2 shows the effect of various treatments to reduce contamination. The air is a primary source of contamination of seeds and materials. Seeds of Mormon tea, shadscale, and rabbitbrush prepared either in the open air or under the hood were 93 to 100 percent moldy, since all seeds had been exposed to the air. Five percent of winterfat seeds became moldy when placed in dishes under a hood. Why the amount of mold obtained from winterfat seeds increased with treatments is unknown, although since winterfat seeds germinate rapidly, molds are not a problem with this species. Treatments of Mormon tea, shadscale, and rabbitbrush were effective, except rinsing Mormon tea with tap water. A chi-square test for independence showed effective treatments to be significant at a level greater than 99 percent.

ALTERNATIVES

Since contamination is a result of a complex of sources and varies among species and seed lots, various measures can be undertaken to reduce mold as experience with seed susceptibility is gained. Working under a hood will by itself reduce mold from the air, but the seeds themselves remain a source of contamination, both internally and as a result of exposure to air during collection and storage. Reducing air

1/ We acknowledge the assistance of Dr. J. J. Ellis, Research Mycologist at the Northern Regional Research Center, in identifying this fungus.

circulation by: (1) blocking vents and fans; (2) closing doors; and (3) using a hood to cover materials, will restrict molds from contaminating treated materials and seeds. Sterilizing vermiculite, blotter paper, water, and dishes will eliminate molds from these materials. It is difficult to reduce the seed source contamination. Nystatin, rinsing, and sodium hypochlorite reduce the levels of mold when the work is done under a hood. These treatments have no apparent effect on germination.

Distributing seeds in the dishes so that they are not in contact with each other will reduce the spread of molds between infected and noninfected seeds. None of the treatments will completely eliminate molds, but any or all of the alternatives will reduce their levels.

Table 1. Number of plates containing cultures from different sources.

Vermiculite	Untreated blotter paper	Treated blotter paper	Untreated Seed	Treated Seed	Vent	Air
2 Bacteria	4 Trichoderma sp.	10 no growth	Sage: 7 no growth	Sage: 6 no growth	1 Trichoderma sp.	During sage counting: 1 Trichoderma sp. 1 Penicillium sp. 1 Stachybotrys sp. 1 Bacterium 1 Bacterium & Penicillium sp.
1 Penicillium sp.	1 R. arrhizus		Shadscale: 3 Penicillium sp. 1 Bacterium 1 unidentifiable fungus 2 no growth	Shadscale: 7 no growth	1 Penicillium sp.	
1 Rhizopus arrhizus 4 no growth	6 no growth					
			Mormon tea: 12 R. arrhizus 1 Pencillium sp.	Mormon tea: 2 R. arrhizus 1 Penicillium sp. 1 unidentifiable fungus 6 no growth		During Mormon tea counting: 1 Trichoderma sp. 4 no growth
			Winterfat: 3 Penicillium sp. 1 R. arrhizus 1 R. arrhizus & 1 Penicillium sp. 1 Bacterium & Penicillium sp. 1 unidentifiable fungus	Winterfat: 5 Penicillium sp. 2 no growth		

Table 2. Effect of seed treatment on mold.

Mormon tea		Shadscale		Rabbitbrush		Winterfat	
Treatment	% moldy seeds	Treatment	% moldy seeds	Treatment	% moldy seeds	Treatment	% moldy seeds
No treatment (open air)	93 (140/150)	No treatment (open air)	100 (150/150)	No treatment (open air)	97 (145/150)	No treatment (under hood)	5 (7/150)
No treatment (under hood)	93 (140/150)	Tap water rinse	27 (40/150)	Tap water rinse	80 (120/150)		
Tap water rinse	93 (140/150)						
Nystatin poured	80 (120/150)	Nystatin poured	16 (24/150)	Nystatin poured	83 (125/150)	Nystatin poured	90 (137/150)
Nystatin immersed	77 (115/150)					Nystatin immersed	95 (142/150)
30 sec. chlorox immersed	50 (75/150)					30 sec. chlorox immersed	90 (135/150)